Project number 27

Characterization of a dNTP Supply Complex and its functionality in DNA damage repair

［1］Research group
Principal Investigator (PI)：
Lene Juel Rasmussen
(University of Copenhagen)
Host researcher at IDAC：
Akira Yasui
(IDAC Tohoku University)
Co-investigator：
Shinichiro Kanno (IDAC Tohoku University)

Expenditure report of research funds：
Consumables 130,000 YEN

［2］Research setup
The molecular and functional characterizations were conducted in the Rasmussen group at University of Copenhagen. The mass spectrometry was conducted in the Akira group at Tohoku University. The collaborating groups have had frequent communication to discuss experimental design and results.

［3］Research outcomes
（3－1）Results
Epigenetic modification of chromatin, including histone methylation and acetylation, plays critical roles in eukaryotic cells and has significant impact on chromatin structure/accessibility, gene regulation and susceptibility to aging, neurodegenerative disease, cancer, and other age-related diseases. TIP60/KAT5 is a major histone acetyltransferase with diverse functions in eukaryotes, and it has been linked to age-related diseases such as cancer, Alzheimer's disease and other neurological diseases.

As the DNA building blocks, the deoxynucleoside triphosphate (dNTP) plays critical roles in proper cell functioning. The imbalance of dNTP levels usually leads to inappropriate DNA replication and insufficient DNA damage repair, which accelerate genomic instability and aging. However, the molecular mechanism behind the dNTP supply at DNA damage sites coupled to DNA repair remains largely elusive in human cells. So, we hypothesize that there is a dNTP supply complex (DSC) generating dNTPs, to facilitate the DNA repair at the local DNA damage sites. To characterize the proteins in DSC, we investigated the histone acetyltransferase TIP60, ribonucleotide reductase (RNR), and serine hydroxymethyltransferases (SHMT1 and SHMT2), all of them are involved in DNA repair and/or nucleotide metabolism. TIP60 and p53R2 (subunit of RNR) protein expressions are upregulated upon UV treatment whereas gene expression is decreased suggesting that the upregulated protein expression of TIP60 and p53R2 is caused by increased protein stability. Further investigation showed that UV irradiation induces SHMT1 translocation to the nucleus and increases TIP60 foci formation in the nucleus. Furthermore, both SHMT1 and TIP60 are recruited to laser-induced DNA damage sites in the nucleus. We also identified specific complex formations, SHMT1-

SKAP2, and SHMT2-REV1. Taken together, we show that upon DNA damage, SHMT1-SKAP2 and SHMT2-REV1 complexes are formed and that TIP60 and SHMT1 are recruited to the DNA damage sites presumably for DNA repair by generating dNTPs. Our studies also show that RNR2 does not interact with SHMT2 and SHMT1 However, RNR2 interacts with ACIN1, SRRM2 and PABP4, which are all proteins building Nuclear speckle. Along these lines SC35, a nuclear speckle protein, co-localizes with RNR2 in H2O2 treated cells. Our study provides new insights into de novo dNTP synthesis for DNA repair at DNA damage sites (Figure)

（3－2）Future perspectives

TIP60 plays a role in multiple cellular pathways, including autophagy, proteasome-dependent protein turnover, RNA transcription, DNA repair, circadian rhythms, learning and memory and other neurological functions. TIP60 also plays a role in processing APP, participates in the AFT complex, and interacts with ATXN1. While speculative at present, TIP60 may be a useful therapeutic target for AD, HD, PD and other aging-related diseases, such as cancer. The roles of TIP60 in normal and pathological aging, oncogene-induced senescence (OIS) and other neurodegenerative disorders remain largely unknown. Therefore, this project can easily development into larges projects. Given the broad field of interest international conferences will likely be relevant.

In humans, TIP60 isoforms are expressed in a broad range of cells and tissues at moderately low levels; however, isoform-specific and cell-type-specific functions of TIP60 are not well characterized. Therefore, additional studies on the cell type- and tissue-specific expression of specific TIP60 isoforms are needed and could provide valuable insight into TIP60 biology. It would also be useful to conduct detailed studies of TIP60 expression in post-mortem brain specimens, cerebrospinal fluid (CSF) and blood, and skin fibroblasts from AD patients. Studies of TIP60 expression and activity in reprogrammed induced pluripotent stem cells (iPSCs) from AD patients, as well as systematic screens for novel substrates of TIP60 would also be valuable for guiding future efforts to exploit TIP60 as a therapeutic target. Therefore, exchange with overseas researchers, collaborative research effect, expansion of researcher network as well as development of young researchers will be highly relevant.

［4］List of Papers

Li Z, Rasmussen LJ. 2020. TIP60 in Aging and Neurodegeneration. Ageing Res Rev. Dec;64:101195. doi: 10.1016/j.arr.2020.101195. Epub 2020 Oct 19.

We are preparing following manuscript for publication:

Characterization of a TIP60-recruited dNTP Supply Complex and Its Functionality in DNA Damage Repair

Zhiquan Li1, Shinichiro Kanno2, Akira Yasui2; Peng Song1, Sharath Anugula1; Claus Desler1, Lene Juel Rasmussen1§

1Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark
2Institute of Development, Aging and Cancer (IDAC), Tohoku University, Japan

§ Corresponding author

Figure: